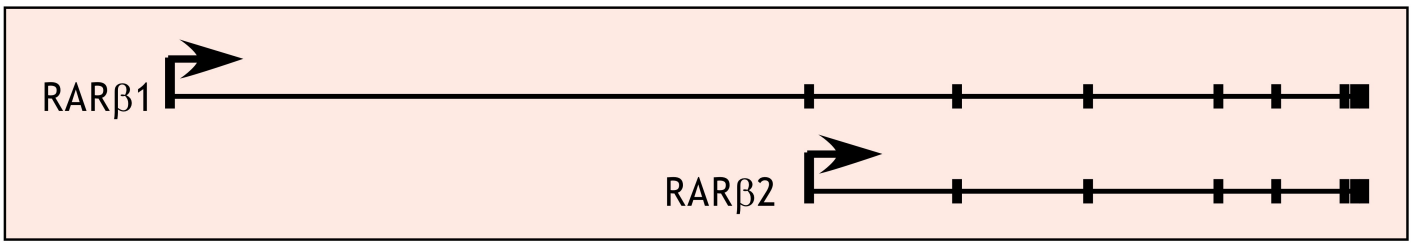
A. Exon Structure



B. N-Terminal Variable Region

>Xl_RARβ1.S (Chr. 6)
M**K**TSSRSC**SG**PAVN**G**HMNHYPASHYPFLFPPVIGGLSLPALHGLHGHPPTSGSSTPSPA**A**

>Xl_RARβ1.L (Chr. 6)
M**T**TSSRSC**PV**PAVN**R**HMNHYPASHYPFLFPPVIGGLSLPALHGLHGHPPTSGSSTPSPA**T**

>Xl_RARβ2.S; Xl_RARβ2.L (Chr. 6)
MFDCMDVLAVSPGQMLDFYTASPSSCMLQEKALKACFSGLAQTEWQHRHSAQS

C. Shared Region

Xl_RARβ1/2.S_Chr. 6 IETQSTSSEDLVPSPPSPPPPPR**V**YKPCFVCQDKSSGYHYGVSACEGCKGFFRRSIQKNMVYTCHR
Xl_RARβ1/2.L_Chr. 6 IETQSTSSEDLVPSPPSPPPPPR**I**YKPCFVCQDKSSGYHYGVSACEGCKGFFRRSIQKNMVYTCHR

Xl_RARβ1/2.S_Chr. 6 EKNCVINKVTRNRCQYCRLQRCF**Q**VGMSKESVRNDRNKKKKEPSK**I**E**F**IE**N**YEMTAELDDL**A**EKIR
Xl_RARβ1/2.L_Chr. 6 EKNCVINKVTRNRCQYCRLQRCF**E**VGMSKESVRNDRNKKKKEPSK**Q**E**C**IE**S**YEMTAELDDL**T**EKIR

Xl_RARβ1/2.S_Chr. 6 KAHQETFPSLCQLGKYTTNSSAD**Q**RVRLDLGLWDKFSELATKCIIKIVEFAKRLPGFTSLTIADQI
Xl_RARβ1/2.L_Chr. 6 KAHQETFPSLCQLGKYTTNSSAD**H**RVRLDLGLWDKFSELATKCIIKIVEFAKRLPGFTSLTIADQI

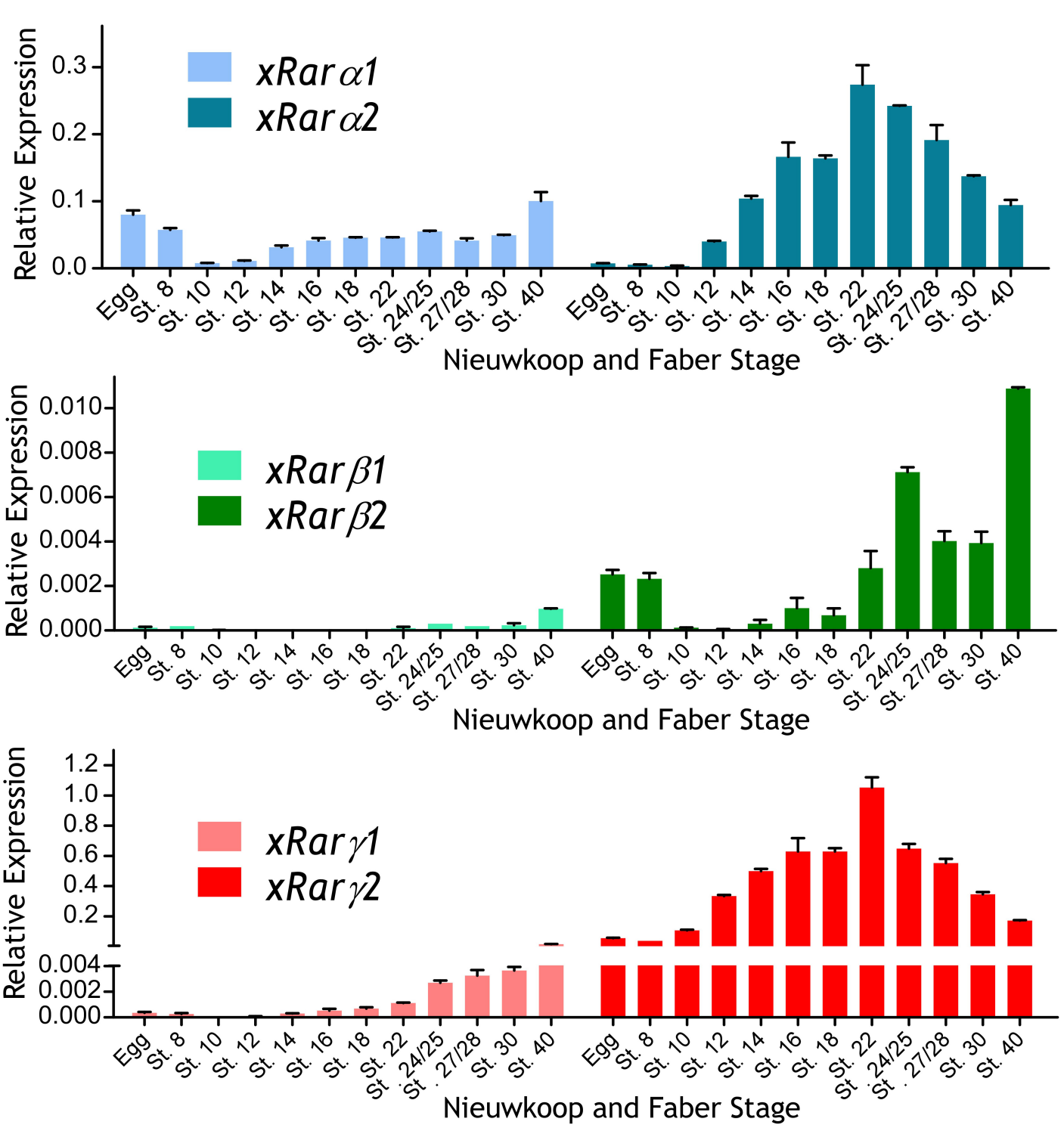
Xl_RARβ1/2.S_Chr. 6 TLLKAACLDILILRICTRFTPEQDTMTFSDGLTLNRTQMHNAGFGPLTDLVFTFANQLLPLEMDDT
Xl_RARβ1/2.L_Chr. 6 TLLKAACLDILILRICTRFTPEQDTMTFSDGLTLNRTQMHNAGFGPLTDLVFTFANQLLPLEMDDT

Xl_RARβ1/2.S_Chr. 6 ETGLLSAICLIC**E**DRQDLEEPAKVDKLQEPLLEALKIYIRKRRPNKPHMFPKILMKITDLRSISAK
Xl_RARβ1/2.L_Chr. 6 ETGLLSAICLIC**V**DRQDLEEPAKVDKLQEPLLEALKIYIRKRRPNKPHMFPKILMKITDLRSISAK

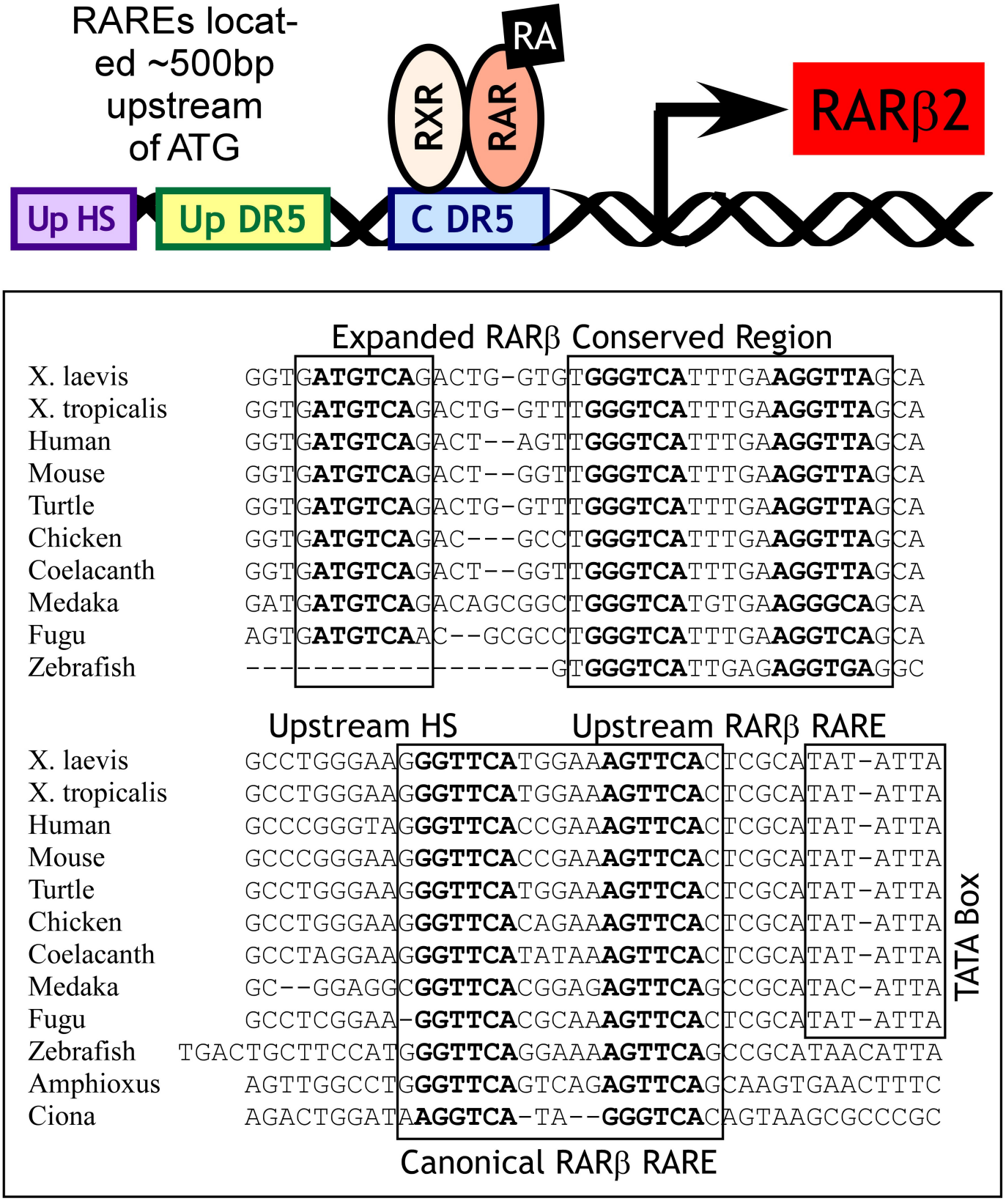
Xl_RARβ1/2.S_Chr. 6 G**A**ERVITLKLEIPGSMPPLIQEMLENSEG**H**EASA**A**SPTEDT**K**EHSH**R**ISPSS-EEE**R**VSKCA**R**VQ
Xl_RARβ1/2.L_Chr. 6 G**T**ERVITLKLEIPGSMPPLIQEMLENSEG**L**EASA**P**SPTEDT**T**EHSH**C**ISPSS**E**EEE**H**VSKCA**Q**VQ

DNA Binding Ligand Binding

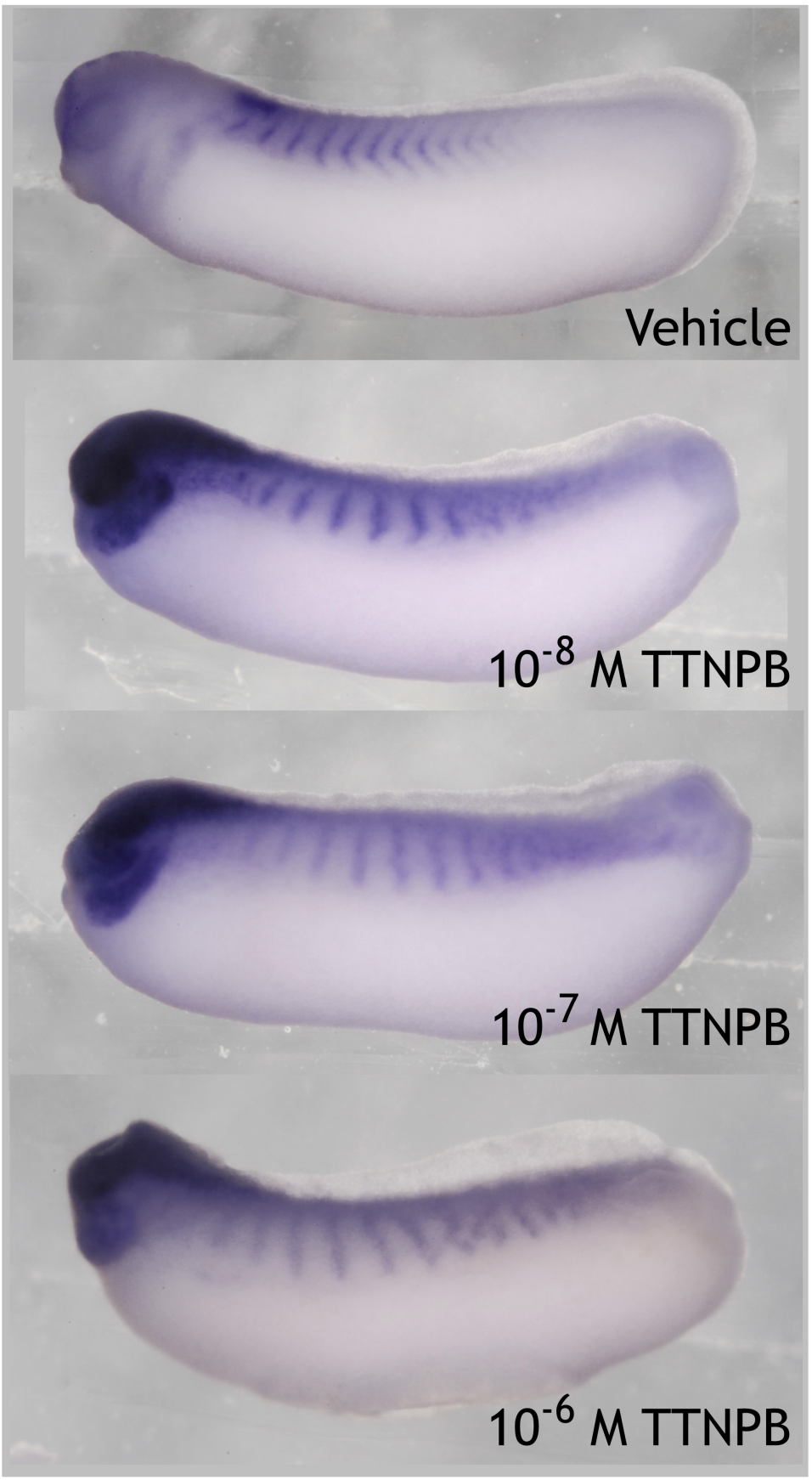
Supplemental Figure S1. Exon structure and protein coding sequence of *Xenopus laevis* RARβ1.S, RARβ1.L, RARβ2.S, and RARβ2.L. Homeologs of RARβ, located on chromosome 6, are denoted by .L and .S notation. **(A, B)** RARβ1 and RARβ2 possess alternative promoters which alter the N-terminal region of the protein (bolded green residues = amino acids not conserved between homeologs). **(C)** Shared region of RARβ1 and RARβ2, with DNA binding domain in red, and ligand binding domain in blue. Bolded amino acid residues are not conserved between homeologs.



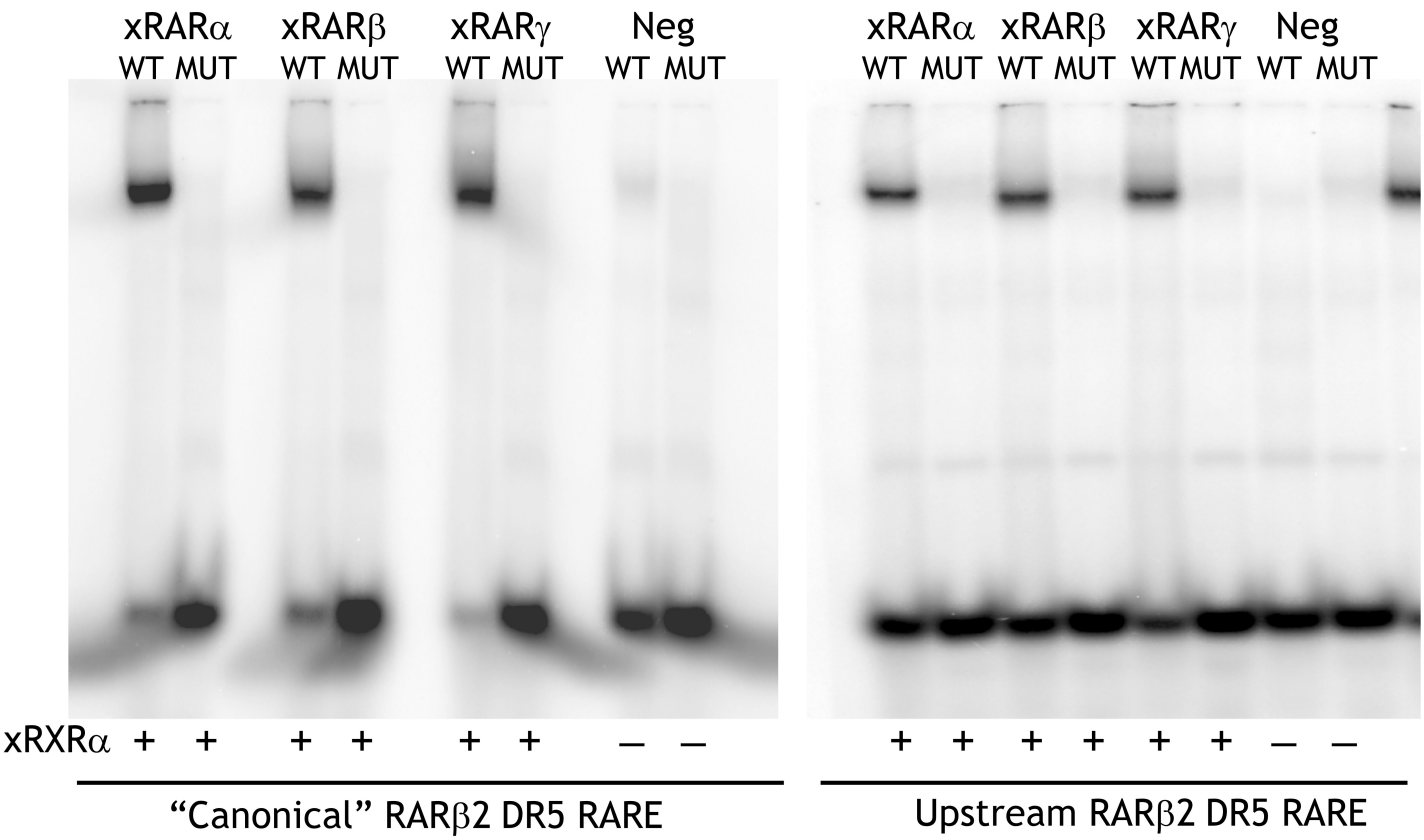
Supplemental Figure S2. Expression of *Rarα1, Rarα2, Rarβ1, Rarβ2, Rarγ1*, and *Rarγ2* across developmental time. QPCR showing *Rarα1, Rarα2, Rarβ1, Rarβ2, Rarγ1*, and *Rarγ2* gene expression over developmental time. Note that *Rarγ1* and *Rarγ2* were previously published in Janesick, et al., 2014, but shown here for comparison purposes. Error bars = S.E.M. The y-axis represents $2^{-\Delta Ct}$ values (adjusted for primer efficiency), normalized to reference gene, *Histone H4*. The y-axis values are comparable for all three graphs.



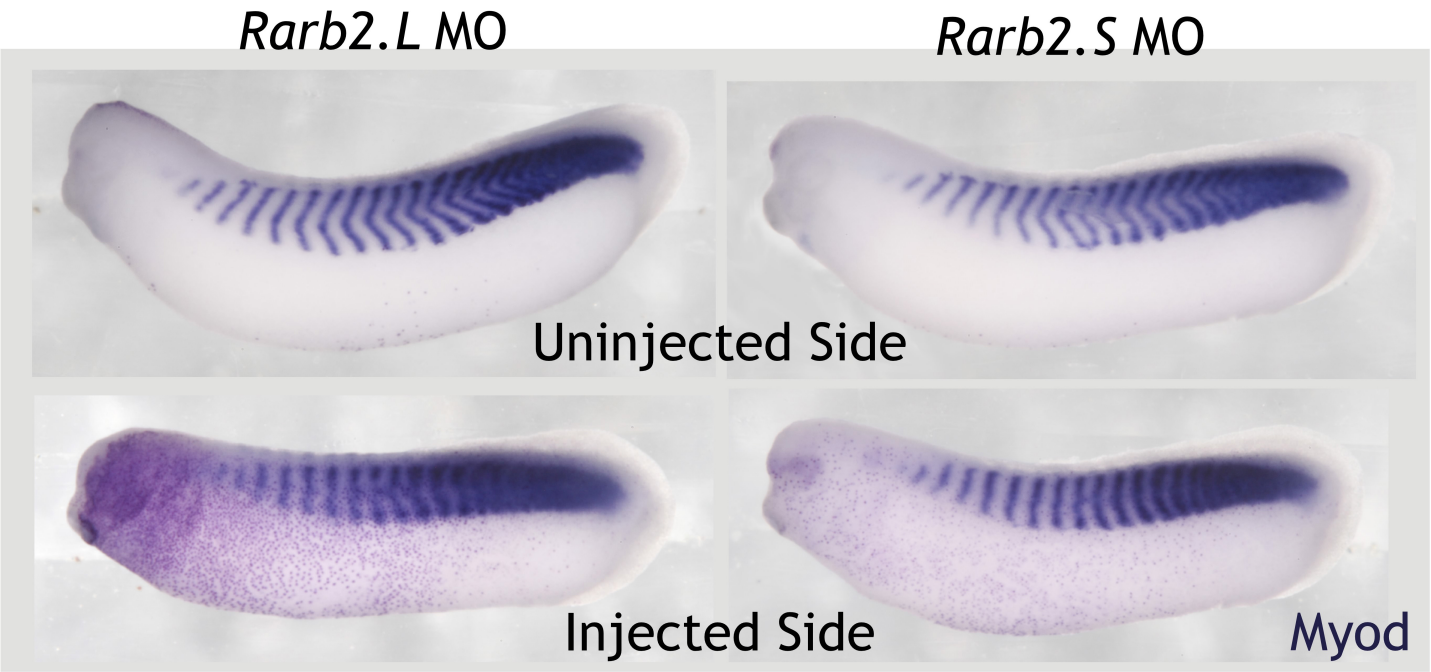
Supplemental Figure S3. *Rarβ2* contains highly conserved retinoic acid response elements (RAREs). Conserved DR5 RAREs and half sites found in the promoter of *Rarβ* in chordate species. Exceptions: zebrafish RARE is found in *raraa* (RARα); Ciona RARE is found downstream, in the first intron. Amphioxus and Ciona lack the upstream HS and upstream RARE. Abbreviations: HS = half site; C = canonical; DR5 = direct repeat 5.



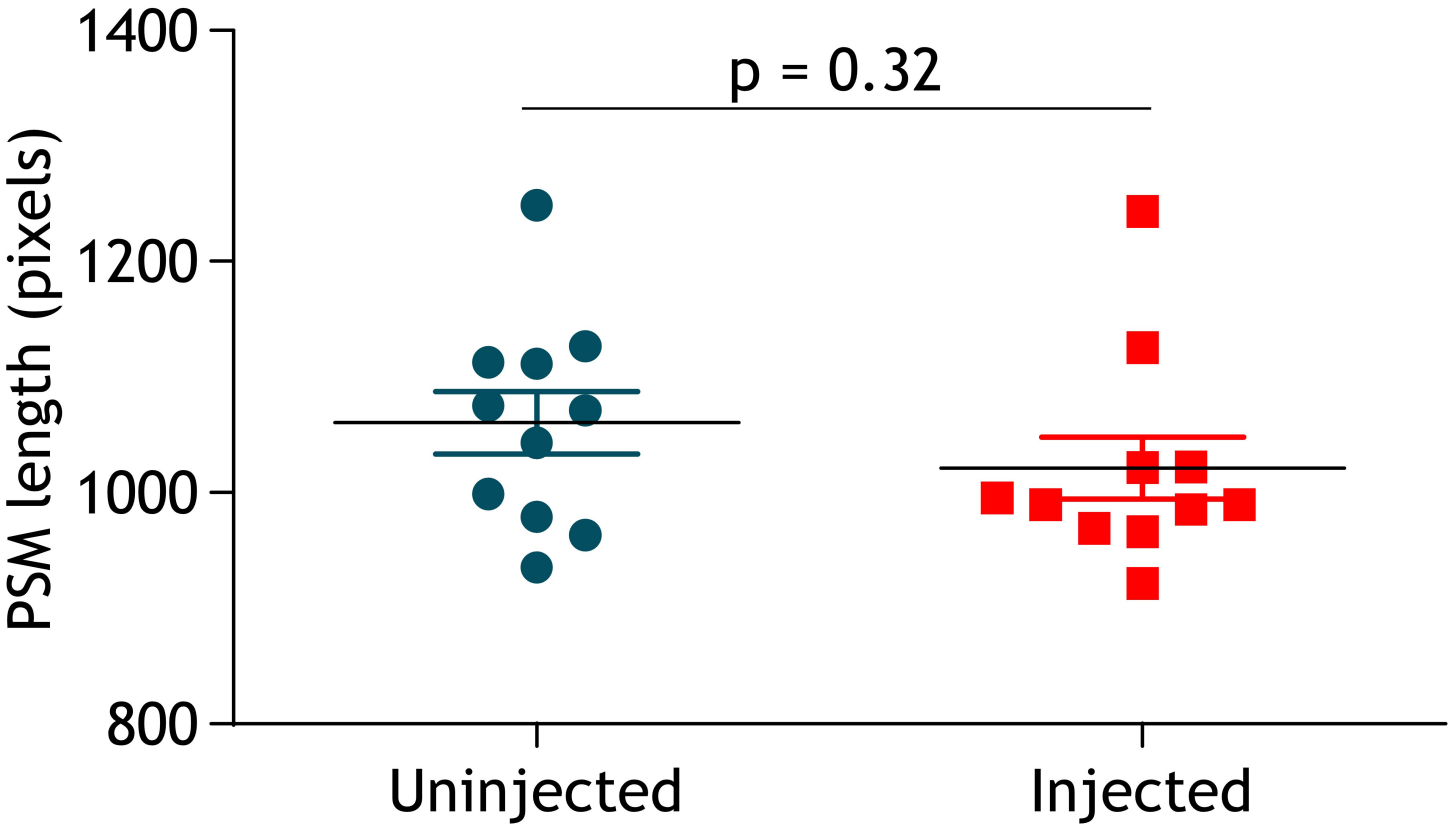
Supplemental Figure S4. *Rarb2* expression is significantly expanded by TTNPB. WISH from embryos treated at stage 6/7 with TTNPB or control vehicle (0.1% EtOH). Embryos are shown in lateral view at tailbud stage 26; anterior is on the left.



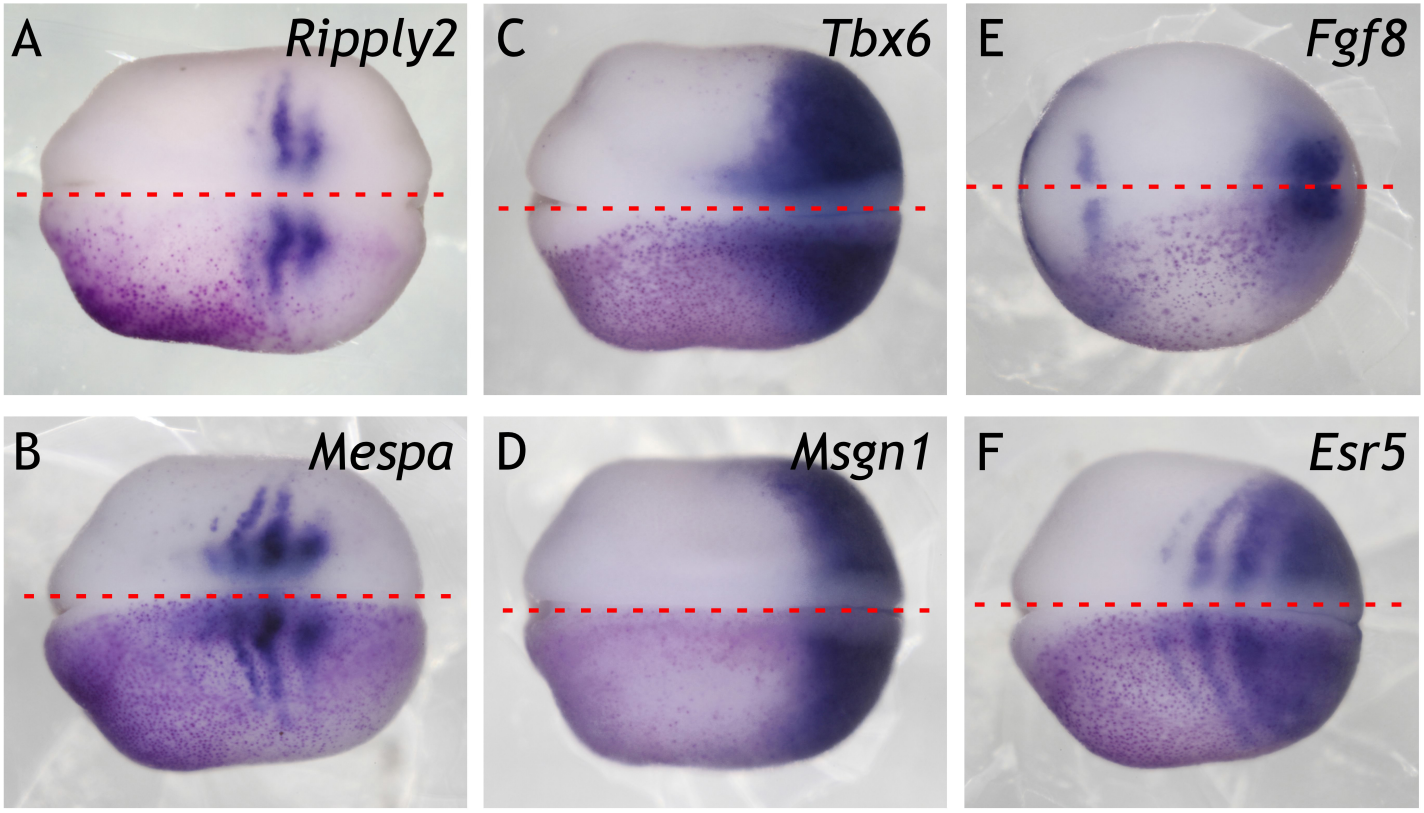
Supplemental Figure S5. Xenopus RARs are capable of binding the canonical and upstream *Rarβ2* direct repeat 5 (DR5) retinoic acid response elements (RAREs). Electrophoretic mobility shift assay with xRAR/xRXR heterodimers was conducted with wildtype and mutated RARE oligonucleotides (**Supplemental material, Table S6**). Negative control was the in vitro translation reaction, minus RNA.



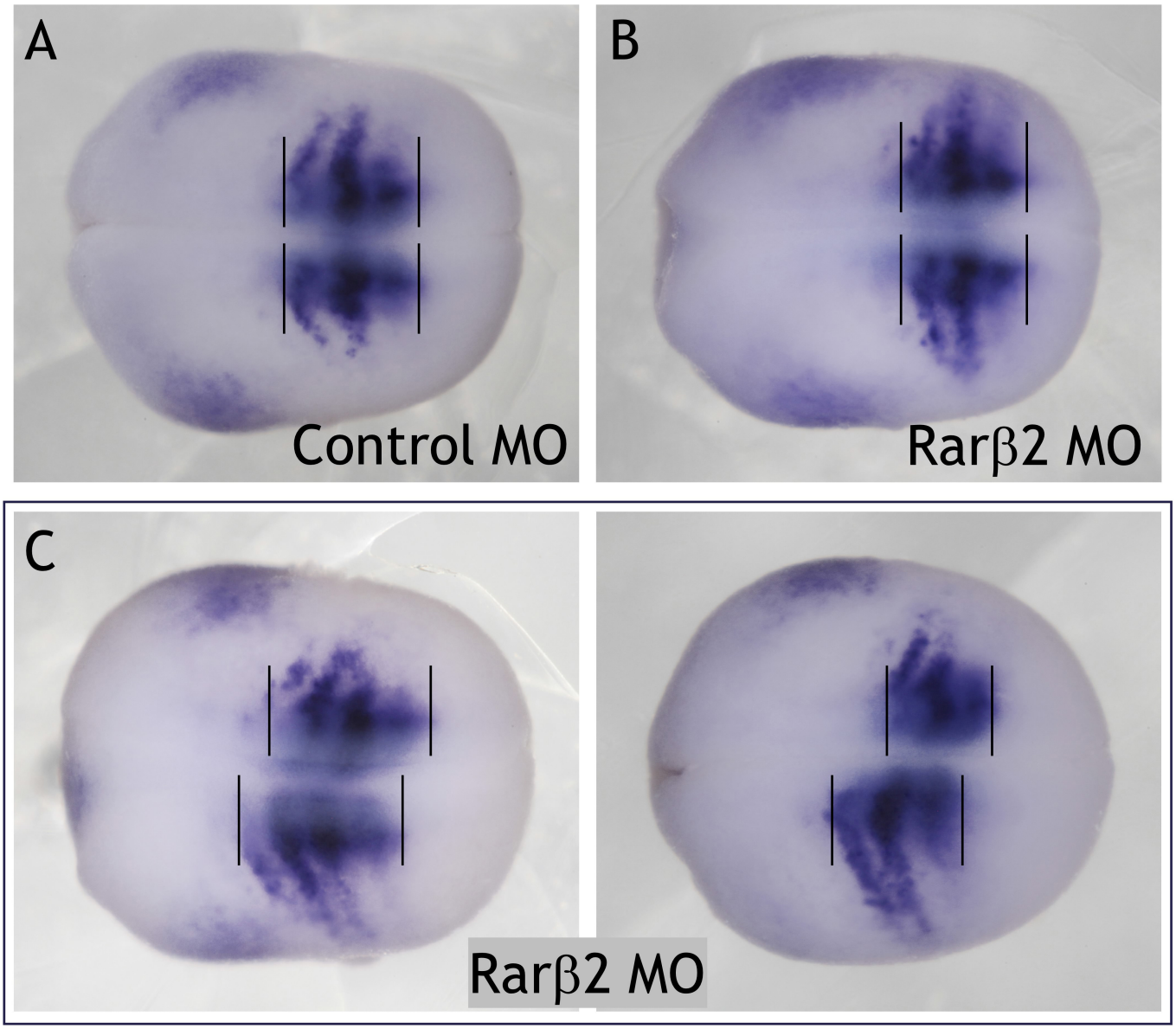
Supplemental Figure S6. Morpholinos targeted against either the .L or .S homeologs of *Rarβ2* result in the same phenotype. Embryos were microinjected unilaterally at 2- or 4-cell stage with 52 ng *Rarβ2.L* MO or 52 ng *Rarβ2.S* MO. Two lateral sides of the same embryo are shown at stage 26; anterior on the left. Injected side is indicated by magenta *β-gal* lineage tracer. *Rarβ2.L* MO or *Rarβ2.S* MO disrupt and disorganize the chevron-shaped somite morphology marked by *Myod*.



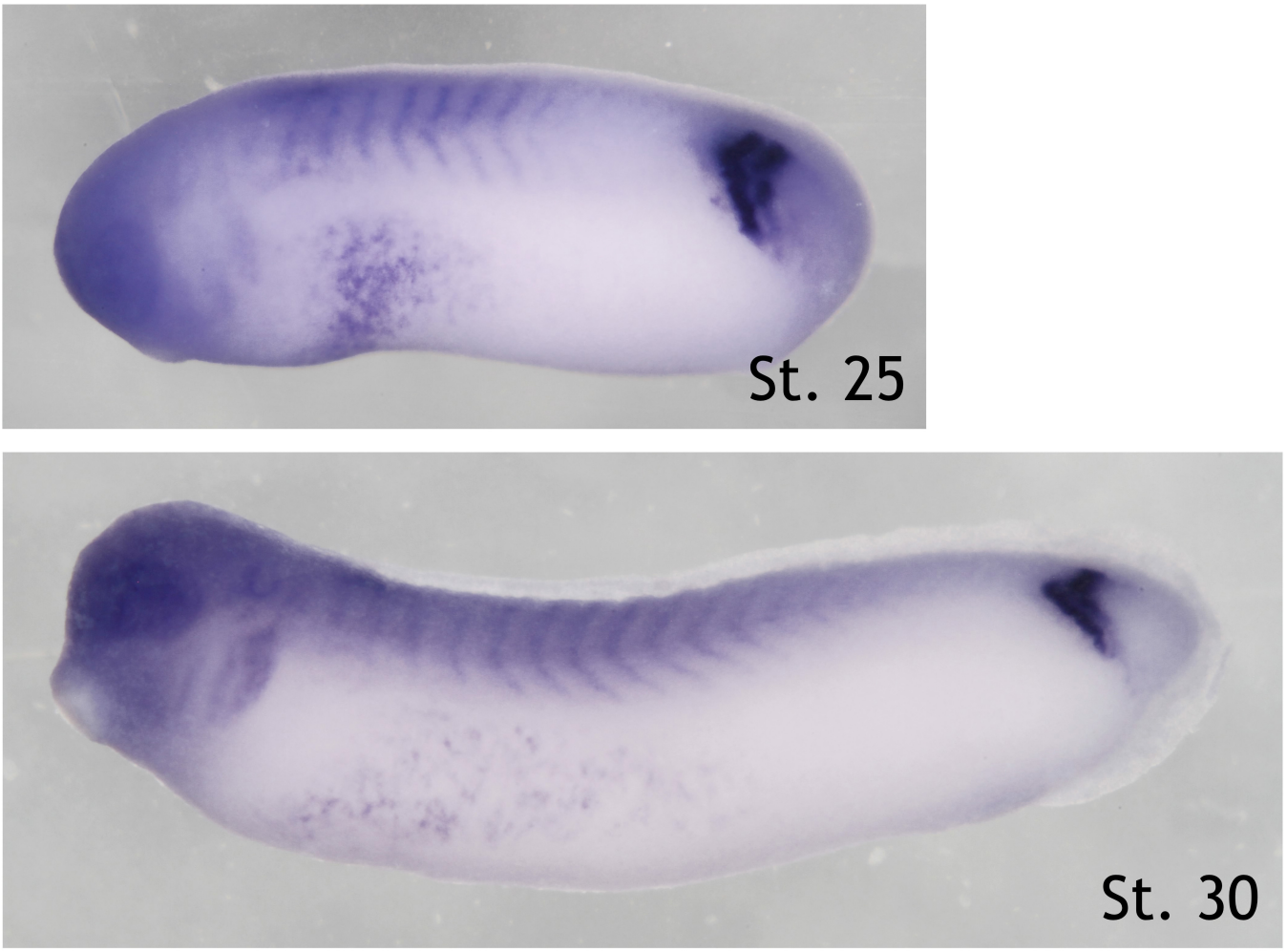
Supplemental Figure S7. Size of the unsegmented PSM is unchanged in Rarß2 MO-injected embryos. Embryos were microinjected unilaterally at 2- or 4-cell stage with 26 ng *Rarß2.L* + 26 ng *Rarß2.S* MOs do not alter the size of the PSM, as indicated by unsegmented *Myod* expression. PSM length is quantitated using ImageJ (units are distance in pixels); each data point represents one embryo. Statistics were conducted in GraphPad Prism v5 using a paired t-test.



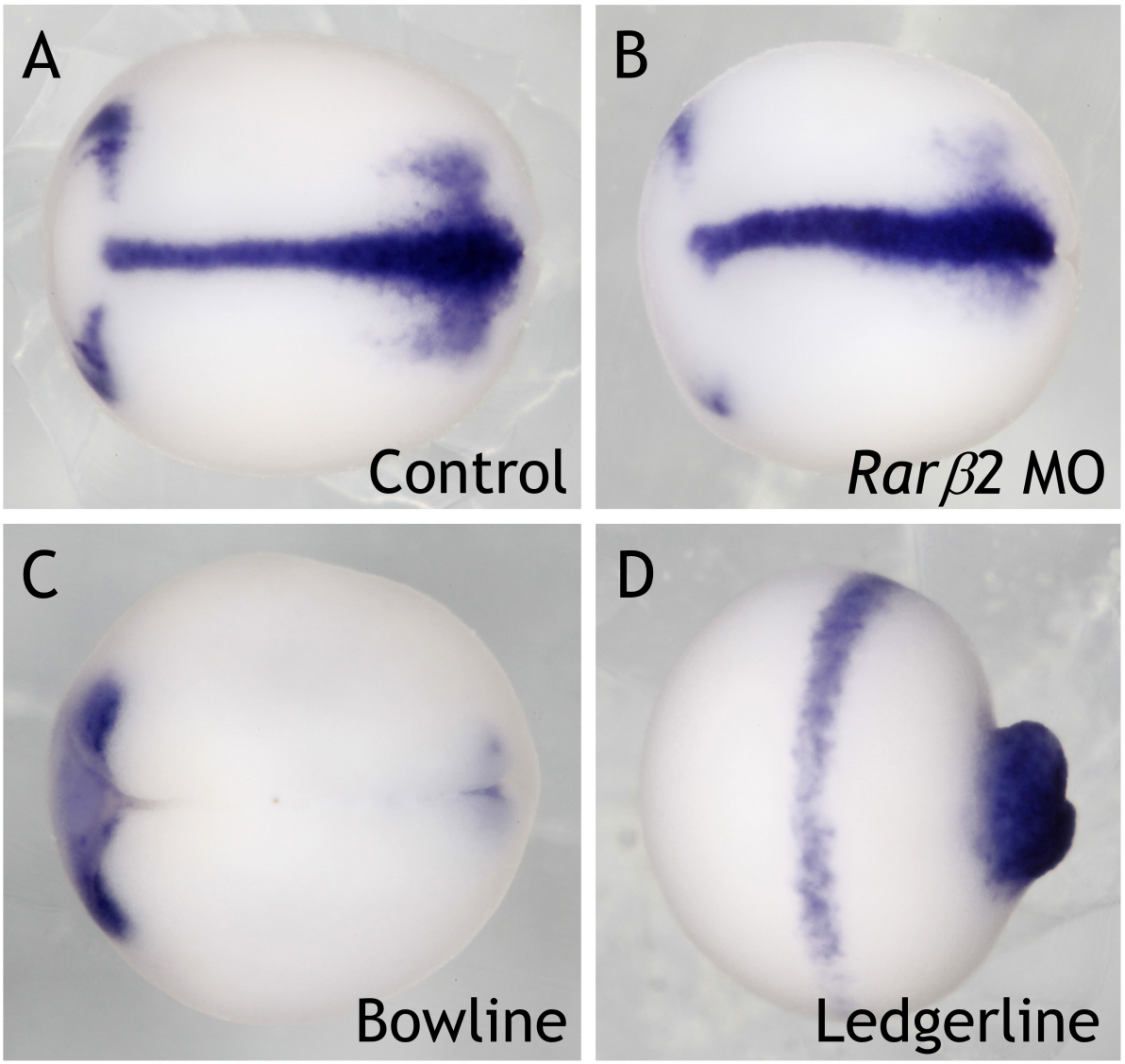
Supplemental Figure S8. Control MO-injected embryos corresponding to Figure 6. **(A-F)** Embryos were injected unilaterally at 2- or 4-cell stage with 52 ng Control MO. Injected side is indicated by magenta ß-gal lineage tracer. Neurula stage embryos shown in dorsal view with anterior on left. Control MO does not alter expression of somitomere markers (A) *Ripply2* (13/15 embryos) and (B) *Mespa/Thyl2* (12/16). The expression domains of presomitic mesoderm markers *Tbx6* (C), *Msgn1* (D), *Fgf8* (E), and *Esr5* (F) are also unaltered (*Tbx6*: 16/17 embryos; *Msgn1*: 21/21; *Fgf8*: 16/18; *Esr5*: 19/20).



Supplemental Figure S9. Somite asymmetry is observed in *Rarβ2* MO-injected embryos. (A-C) Embryos were injected bilaterally at 2-cell stage with **(A)** 52 ng Control MO or **(B, C)** 26 ng *Rarβ2.1* MO + 26 ng *Rarβ2.2* MO. Neurula stage embryos shown in dorsal view with anterior on left. *Rarβ2* MOs resulted in either **(B)** normal somite symmetry (17/25 embryos) or **(C)** abnormal somite asymmetry (8/25). Control MO **(A)** showed normal somite symmetry (22/23)



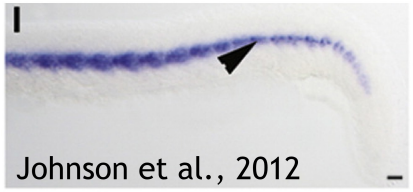
Supplemental Figure S10. Double WISH of *Rarβ2* and *Ripply2*.



Supplemental Figure S11. Notochord (*Xnot*) defects observed in *Rarβ2* MO-injected and *Ripply2*-overexpression embryos. (A-D) Embryos were injected bilaterally at 2-cell stage with **(A)** 52 ng Control MO or 0.5 ng Control *mCherry* mRNA (one representative control shown here), **(B)** 26 ng *Rarβ2.1* MO + 26 ng *Rarβ2.2* MO, **(C)** 0.5 ng *Bowline* mRNA or **(D)** 0.5 ng *Ledgerline* mRNA. Neurula stage embryos shown in dorsal view with anterior on left. *Rarβ2* MOs resulted in a crooked notochord (10/19 embryos) and shorter body axis (19/19 embryos). *Bowline* overexpression eliminated *Xnot* expression (16/16 embryos) and *Ledgerline* created severe axial defects with diminished *Xnot* expression (23/24 embryos).

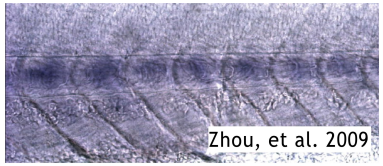
Zebrafish

En1

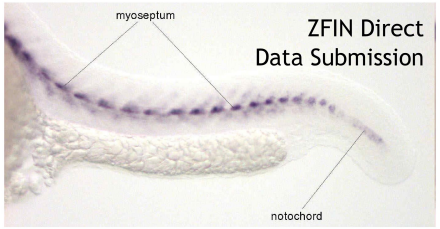


Cxcl12a

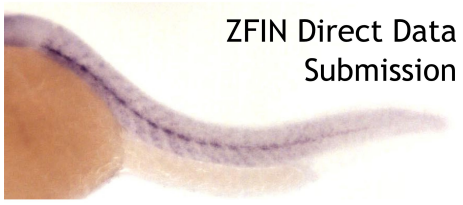
Plce1



Wnt11

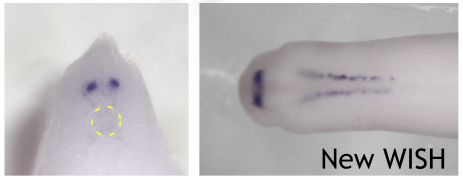


Hhatl



Xenopus

En1



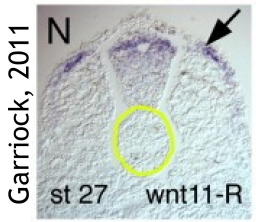
Cxcl12a

Probe designed.
No specific signal.

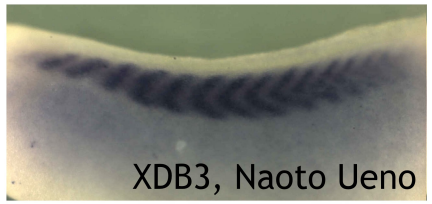
Plce1

No Xenbase data
Did not test because
FPKM was <1

Wnt11

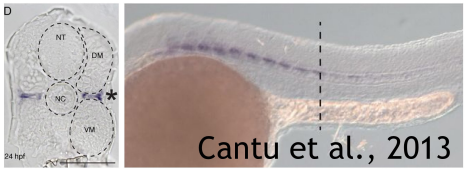


Hhatl

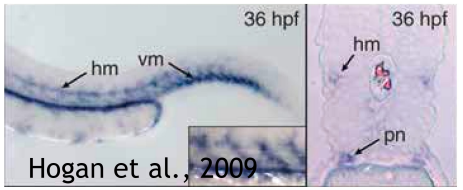


Zebrafish

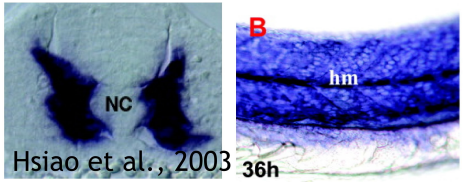
Notum



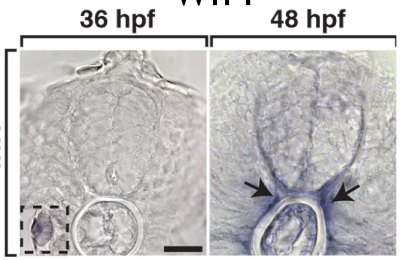
Ccbe1



Mybpc1

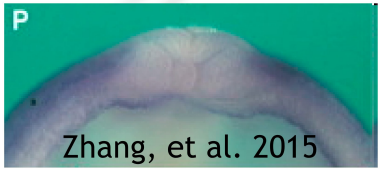


Wif1



Xenopus

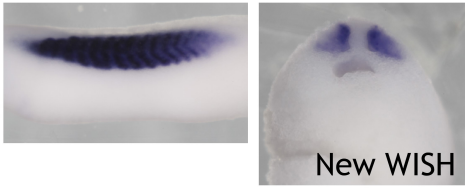
Notum



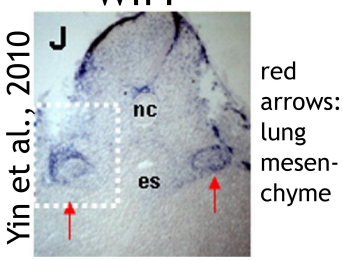
Ccbe1

No Xenbase data
Did not test because
FPKM was < 0.1

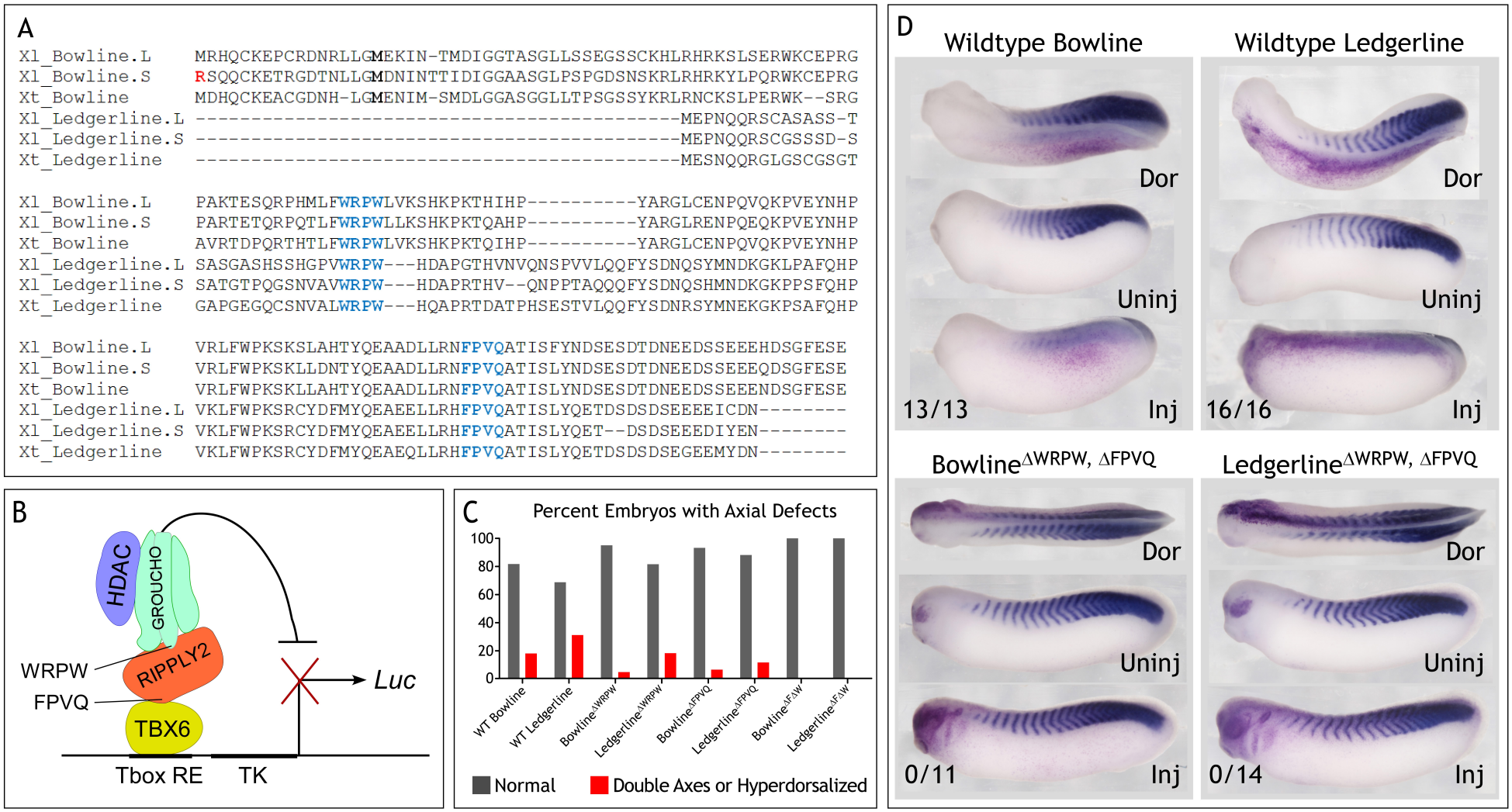
Mybpc1



Wif1



Supplemental Figure S12. Comparison of mysoseptum and adaxial "pioneer" markers in zebrafish versus Xenopus. New WISH indicates that a probe was designed specifically for this paper, and was not published previously or available on Xenbase. FPKM = Fragments Per Kilobase of transcript per Million mapped reads (data not shown).



Supplemental Figure S13. (A) MAFFT multiple alignment of Xenopus *Ripply2* proteins Bowline and Ledgerline. Xl = Xenopus laevis; Xt = Xenopus tropicalis. All sequences were obtained from Xlv9.1 or Xtv9.0 genomes on Xenbase (Karpinka et al., 2014). **(B) Diagram of T-Box response element (RE) TK-luciferase (luc) reporter and *Ripply2* protein interactions.** The Brachyury palindromic element is present in two copies, preceding the TK minimal promoter and luciferase. **(C) Quantitation of axial defects due to Ripply2 overexpression.** Embryos were microinjected unilaterally at 2- or 4-cell stage with 0.5 ng *Ripply2* wildtype or mutant mRNA (ΔWRPW = *Ripply2*$^{WRPW->AAAA}$; ΔFPVQ = *Ripply2*$^{FPVQ->AAAA}$; ΔWΔF = *Ripply2*$^{WRPW->AAAA,\ FPVQ->AAAA}$). **(D) *Ripply2* blurs and abolishes *Myod* expression in the somites.** Embryos were microinjected unilaterally at 2- or 4-cell stage with 0.5 ng *Ripply2* wildtype or mutant mRNA. Injected side is indicated by magenta *β-gal* lineage tracer. Embryos are shown in lateral view at stage 26; anterior on the left. Single mutant mRNA not pictured, but scored for *Myod* blurring and knockdown: *Bowline*$^{\Delta WRPW}$ (3/13 embryos); *Ledgerline*$^{\Delta WRPW}$ (18/18); *Bowline*$^{\Delta FPVQ}$ (4/14 blurred, 4/14 disorganized); *Ledgerline*$^{\Delta FPVQ}$ (2/22)

Supplemental Table S1 (Morpholinos)

MO	Sequence (5'→3')
Rarβ2.S	AGC TAA TAT TGT GTA TAG GGG GGG A
Rarβ2.L	CTT TAC AGA ATA TCA GCG AAA GTG C
Rarγ1.L/S	GCT GTT TGC CAT TGC CTT GTT CTA
Rarγ2.L/S	TTC CAT GCA GTC ATA CAT TTT GGG
Rarα1	GCT CCA AAC GCA CTT CTA CTC CCT C
Rarα2.S	CTG AAA TCC AAA CTG ACC ATA GAG T
Rarα2.L	ATC CAA AGG AAG GTG AGT GTG TGT G

Supplemental Table S2 (Probe Design)

Probes with T7 Adapters

Primer	Sequence (5'→3')
F (*Rarβ1*):	GAT AAT ACA TCA TTC TTT GCC TCC CTG G
R (*Rarβ1*):	taa tac gac tca cta tag ggC TCT GGG TTT CGA TGG TTG CTG
F (*Rarβ2*):	CAG GAA TTT AGA TGC ATT TTG CCT GG
R (*Rarβ2*):	taa tac gac tca cta tag ggC CAT TCT GTC TGT GCC AAT CCA C
F (*Rarβ2-Sense*):	taa tac gac tca cta tag ggA GGA ATT TAG ATG CAT TTT GCC TGG
R (*Rarβ2-Sense*):	CCA TTC TGT CTG TGC CAA TCC AC
F (*Ripply2*):	GCA AGT GGT TTG CCA AGT CC
R (*Ripply2*):	taa tac gac tca cta tag ggT CAA ATC CAG AGT CTT GTT CCT CC
F (*Mespa/Thyl2*):	ACA CTT AAA CCA GAG TCT TTC ACC T
R (*MespaThyl2*):	taa tac gac tca cta tag ggA TCT GAA GCT TTG CCT TCA GTG G
F (*Mesogenin*):	AAT GGA AGA GGA CTA TGC CTT GAG
R (*Mesogenin*):	taa tac gac tca cta tag ggT CTT GGA GCA CTG GAG AAG GT
F (*Tbx6*):	GGC ACC TCC TAC ACG ATG AGA C
R (*Tbx6*):	taa tac gac tca cta tag ggC TCC TCT TCC TGT TCC TGT TCC A
F (*MyoD*):	CAC TGC GGG ACA TGG AAG TC
R (*MyoD*):	taa tac gac tca cta tag ggG TAT TGC TGG GAG AAG GGA TGG T
F (*Esr5*):	TCC AGG AAG ATC CTC AAA CCG
R (*Esr5*):	taa tac gac tca cta tag ggC TCC ATA TGT ACA ATG GCG GCT G
F (*Cxcl12*):	GCT CTG CTC TCC ATC CTG CT
R (*Cxcl12*):	taa tac gac tca cta tag ggC TTT CTC CAG GTA CTC C
F (*Mybpc1*):	GGT TGA AAG GCA AAT GGA TGG AC
R (*Mybpc1*):	taa tac gac tca cta tag ggA GGT TCT CTG ACA AAC AGC
F (*En1*):	TCT TCA TAG ACA ACA TTC TCC GGC
R (*En1*):	taa tac gac tca cta tag ggT GGT TGT ACA GTC CCT GAG C
F (*Tbx3*):	TGT ATA TCC ATC CAG ACA GCC CG
R (*Tbx3*):	taa tac gac tca cta tag ggT AGA TTC GCC TGT GTC CG

Supplemental Table S3 (QPCR)

Primer	**Sequence (5'→3')**
F (*xRARα1*):	CCA CAT ATG TTG GGG GGT ATG TC
R (*xRARα1*):	GAT TCT GGG GAG CGG TGG T
F (*xRARα2*):	CCA CTC AAT TGA GAC TCA GAG CAC
R (*xRARα2*):	CTC TTG TCC TGA CAC ACA AAG CA
F (*xRARβ1*):	TTT CCT CCT GTC ATT GGT GGA CTC
R (*xRARβ1*):	GCT CTG GGT TTC GAT GGT TGC
F (*xRARβ2*):	CAA ATG CTG GAT TTC TAC ACT GCG
R (*xRARβ2*):	GTG TTG CCA TTC TGT CTG TGC C
F (*xRARγ1*):	AGA ACA AGG CAA TGG CAA ACA G
R (*xRARγ1*):	GCA AGT ACT TCA AAT GGT GGA GAT C
F (*xRARγ2*):	GTA GAA ACA CAA AGT ACC AGC TCG
R (*xRARγ2*):	CCG TAG TGA TAA CCT GAA GAC TTG T
F (*Histone H4*):	GAT AAC ATC CAG GGC ATC AC
R (*Histone H4*):	TAA CCT CCG AAT CCG TAC AG
F (*Eef1a1*):	CAT CTC GCC CAA CCG ATA AGC
R (*Eef1a1*):	TTT AAT GAC ACC AGT CTC CAC ACG

Supplemental Table S4 (pCDG1 Expression Constructs, *Ripply2* Two-Fragment PCR)

Primer	**Sequence (5'→3')**
F (pCDG1-*xTbx6*):	cag ata cca tgg AAT ACC ACT CTG AGC TCT TCC AGC AGT AT
R (pCDG1-*xTbx6*):	act agt gga tcc TTA CTA TCA CAT CCA GCC CCC C
F (pCDG1-*xTbx3*):	cag ata cca tgg ATT TAC CCA TGA GAG ATC CAG TAA TTT CAG G
R (pCDG1-*xTbx3*):	act agt gga tcc TTA TCA GTC AGG GGA ACC GCT C
F (pCDG1-*Ledgerline*):	cag ata cca tgg AGC CGA ATC AAC AGC GGA G
R (pCDG1-*Ledgerline*):	act agt gga tcc TTA ATT TTC ATA AAT GTC TTC CTC TTC AG
F (pCDG1-*Bowline*):	cag ata cca tgg ACA ACA TTA ACA CTA CTA TTG ACA TTT
R (pCDG1-*Bowline*):	act agt gga tcc TTA TTC AGA TTC AAA TCC AGA GTC TTG T
F (pCDG1-*Ledgerline*_WRPW_B):	ATG TGC AGC TGC AGC CAC TGC CAC ATT GCT GCC CT
R (pCDG1-*Ledgerline*_WRPW_C):	GTG GCT GCA GCTG CAC ATG ATG CCC CTA GGA CCC AT
F (pCDG1-*Ledgerline*_FPVQ_B):	AGC TGC AGC TGC AGC ATG TCT CAA TAA TTC CTC GGC TTC CT
R (pCDG1-*Ledgerline*_FPVQ_C):	ACA TGC TGC AGC TGC AGC TAC AAT ATC TCT TTA CCA GGA GAC AG
F (pCDG1-*Bowline*_WRPW_B):	AAA GTG CAG CTG CAG CGA ACA ATG TTT GAG GTC TCT GTG TTT C
R (pCDG1-*Bowline*_WRPW_C):	GTT CGC TGC AGC TGC ACT TTT GAA ATC CCA TAA ACC AAA GAC CC
F (pCDG1-*Bowline*_FPVQ_B):	GGC TGC AGC TGC AGC GTT TCT AAG CAA ATC TGC TGC CT
R (pCDG1-*Bowline* FPVQ_C):	AAC GCT GCA GCT GCA GCC ACA ATA TCT CTC TAC AAT GAC TC

Supplemental Table S5 (β-RARE Two-Fragment PCR)

Primer	Sequence (5'→3')
F (pGL3-RARβ2.2_A)	ccc ggg ctc gag GCT CGC TGC TAG TCT TTA AGC TG
R (RARβ2.2_Mut_C-DR5_B)	TGC GAG **TGT TCT** TTC CA**T GTT CC**C TTC CCA GGC TGC TAA CC
F (RARβ2.2_Mut_C-DR5_C)	CTG GGA AG**G GAA CA**T GGA A**AG AAC A**CT CGC ATA TAT TAG GC
R (RARβ2.2_Mut_Up-DR5_B)	CAG GCT GC**T ATT CT**T CAA A**TG TTC C**AC ACC AGT CTG ACA TCA C
F (RARβ2.2_Mut_Up-DR5_C)	CTG GTG T**GG AAC A**TT TGA **AGA ATA** GCA GCC TGG GAA GGG TTC ATG GA
R (RARβ2.2_Mut_HS_B)	TGA CCC ACA CCA GTC **TGT TAT** CAC CAA CTC CCA GGA TTC TCA
F (RARβ2.2_Mut_HS_C)	CTG GGA GTT GGT G**AT AAC A**GA CTG GTG TGG GTC ATT TGA AGG
R (pGL3-RARβ2.2_D)	aat gcc aag ctt CAG CTC ACT TCC TAC TAC TTG TGT

Supplemental Table S6 (β-RARE EMSA)

Primer	Sequence (5'→3')
F (EMSA_RARE_C-DR5)	GGAAG**GGTTCA**TGGAA**AGTTCA**CTCGC
R (EMSA_RARE_C-DR5)	GCGAG**TGAACT**TTCCA**TGAACC**CTTCC
F (EMSA_RARE_Mut_C-DR5)	GGAAG**GGAACA**TGGAA**AGAACA**CTCGC
R (EMSA_RARE_Mut_C-DR5)	GCGAG**TGTTCT**TTCCA**TGTTCC**CTTCC
F (EMSA_RARE_Up-DR5)	GGTGT**GGGTCA**TTTGA**AGGTTA**GCAGC
R (EMSA_RARE_Up-DR5)	GCTGC**TAACCT**TCAAA**TGACCC**ACACC
F (EMSA_RARE_Mut_Up-DR5)	GGTGT**GGAACA**TTTGA**AGAATA**GCAGC
R (EMSA_RARE_Mut_Up-DR5)	GCTGC**TATTCT**TCAAA**TGTTCC**ACACC